

Nutritive and immunological benefits derived from the honey bee gut microbiome are threatened by anthropogenic stressors

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Insect gut microbes have a disproportionate effect on their hosts, including the honey bee, *Apis mellifera* L. The honey bee gut, and that of other species that have been investigated, harbours a specific gut community that is conserved across populations globally. However, this gut community changes between different castes, sexes and life stages, which is largely due to diet and gut physiology. Evidence suggests that a healthy gut community is essential for honey bees to maintain functional immunity and nutrition. Without the four important gut symbionts (*Lactobacillus*, *Bifidobacterium*, *Snodgrassella alvi* and *Gilliamella apicola*), honey bees lack the ability to gain sufficient nutrients, protect themselves against pathogens and prevent intoxication. Unfortunately, honey bee gut symbionts are under threat due to in-hive pesticides, antibiotics and climate change. Therefore, we discourage the overuse of antibiotics and in-hive pesticides, as they could have unforeseen consequences for the honey bee gut microbiota. Instead, we recommend that beekeepers and scientists explore alternative options, such as bolstering honey bee resilience through probiotics.

INTRODUCTION

Insects are more abundant and diverse than any other animal clade. The over one million described insect species occupy many ecological niches and have a far reaching effect on their surroundings (Stork 1988). Their success can be partially attributed to their close association with microorganisms. From fungus gardens in termite mounds to breaking down pollen in bee guts, these complex associations between microorganisms and insects have been around for millennia (Labandeira and Sepkoski 1993). We are regularly reminded of the profound effects microbes have on the host by the burgeoning number of research papers on this subject (Amato 2013; Kwong and Moran 2016). According to search results from Web of Science, the number of published papers on insect gut microbes increased by $\pm 52\%$ per year from 2017 to 2020 (Analytics 2017).

Arguably, the most common and important association between animals and microorganisms occurs in the gut (Lee and Hase 2014), with communities of bacteria, viruses, protozoa, or fungi present in the gastrointestinal tract of the host organism (Hacquard et al. 2015). Gut microorganisms are ubiquitous in most animals and these relationships range from mutualistic and commensalistic to antagonistic, but the former is most common (Hacquard et al. 2015). Many previous studies have investigated the gut microbiome and its effect on animal digestion, immune function and nutrient uptake or quality (Amato 2013; Habineza et al. 2019; Lee and Hase 2014; Pal and Karmakar 2018). However, gut microbial communities are usually complex, containing hundreds of taxa with specific roles and functions, and our ability to study these communities is hindered, especially in mammals, by an inability to identify, manipulate or culture them (Amato 2013; Zheng et al. 2017). As a consequence, focus has shifted to using model organisms with simple gut microbiomes that are easily manipulated, to study these complex communities (Zheng et al. 2018).

Fortunately, the specialised gut microbiome of honey bees (*Apis mellifera* L.) is relatively simple, dominated by only nine bacterial taxa (Engel et al. 2012). The gut microbiota of honey bees share many similarities to that of the mammalian gut microbiome, making them an excellent model for study (Amato 2013; Kwong and Moran 2016; Zheng et al. 2018). The honey bee and human gut community comprise of host-adapted, facultative anaerobes and microaerophiles with extensive strain-level variation and they share similar gut taxa such as *Lactobacillus* and *Bifidobacterium*, passing these on via social interactions (Zheng et al. 2018).

Unfortunately, we are amidst a global extinction crisis. Native bee species, along with other insects, are at risk of eradication (Abudulai et al. 2022; Cardoso and Leather 2019; Goulson et al. 2015; Sánchez-Bayo and Wyckhuys 2019; Weisser et al. 2023). Studies have reported an insect decline of up to 75% in protected areas and similar trends are observed across the globe (Vacharaksa and Finlay 2010). The economic value of insect pollination services to the agricultural industry worldwide is estimated at €153 billion, with honey bees providing the largest proportion of pollinating services (Gallai et al. 2009). Subsequently, the cost to consumer welfare is also estimated at between €190 and €310 billion. These declines not only result in economic losses, but the loss of biodiversity and an important food source for many animals (Hung et al. 2018). Specifically, honey bees face many challenges that beekeepers and scientists are still struggling to control (Genersch 2010; Goulson et al. 2015; Vanbergen 2013). Diseases such as American foulbrood (AFB), Nosema, and Chalkbrood, and parasites such as Varroa mites, all relate to losses of honey bees (Genersch

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2010; Pirk et al. 2014). Honey bees are not only affected by biotic factors, but also abiotic factors such as pesticide overuse, and pollution (Goulson et al. 2015). These effects can be sub-lethal, influencing the fecundity, mortality or development of honey bees (Goulson et al. 2015). Recent studies also suggest that the honey bee gut microbiome is significantly affected by in-hive pesticides and antibiotics (Kakumanu et al. 2016; Raymann et al. 2017). This can have disastrous consequences for honey bee health, as the importance of gut symbionts have become more apparent in recent years (Kwong and Moran 2016).

In this review we discuss the importance of the native honey bee gut microbiota and the potential interactions between gut microbial composition, diseases, pests, pesticides and other stressors. We summarise the diversity within the honey bee gut between different castes, sexes, as well as the pathways of transmission. Particular focus is put on recent research articles that investigate the effects that gut microbes have on both nutrition and immunology. Lastly, we highlight the current threats to honey bees with a compromised gut microbiome, focusing on the southern African region, and provide some recommendations on how to minimise these risks.

STRUCTURE AND MICROBIAL DIVERSITY OF THE HONEY BEE GUT COMPARED TO OTHER SPECIES

The honey bee gastrointestinal tract shares many similarities to that of other insect guts and is composed of three main sections: the foregut, midgut and hindgut (Figure 1) (Engel et al. 2012; Engel and Moran 2013; Kwong and Moran 2016; Snodgrass 1910). The foregut comprises the mouth, oesophagus and crop (Snodgrass 1910). Food enters through the mouth, travels down the oesophagus and ends up in the crop. The crop is often the first site of digestion and is used in trophallaxis, the mutual exchange of regurgitated food or liquids between individuals. The crop is common in most social insects and is hypothesised as one of the keys to the evolution of sociality in insects (Contrera et al. 2010). Surprisingly, this is not the main transmission pathway of gut microorganisms as the foregut harbours few microbes (Figure 1, Figure 2) (Engel et al. 2012; Engel and Moran 2013; Kwong and Moran 2016; Martinson et al. 2012).

The midgut is where digestion and absorption of food primarily occurs (Figure 1) (Snodgrass 1910). As with the foregut, this region of the gut typically harbours few microorganisms (Engel et al. 2012; Engel and Moran 2013; Kwong and Moran 2016; Martinson et al. 2012). This is largely due to the instability caused by shedding of the chitinous lining (peritrophic membrane) in the bee midgut (Engel and Moran 2013). The foregut and midgut lack suitable substrates and environment for most gut microbes to persist (Engel and Moran 2013; Martinson

et al. 2012). Thus, the microorganisms often found in the foregut and midgut are low in abundance or usually associated with the hive environment (Engel et al. 2012). The foregut and midgut are commonly dominated by less numerous Enterobacteriaceae, *Lactobacillus kunkeei*, *Parasaccharibacter apium*, *Bartonella apis* (Alpha-1) and *Gluconobacter*-related taxa (Alpha-2) (Figure 1) (Engel et al. 2012; Engel and Moran 2013; Kwong and Moran 2016; Martinson et al. 2012).

The hindgut comprises the ileum and rectum (Figure 1) (Engel et al. 2012; Engel and Moran 2013; Snodgrass 1910). The hindgut functions in the absorption of salt, water and other important molecules before excretion (Snodgrass 1910). It is also where 95–99% of honey bee gut microbes reside (Kwong and Moran 2016; Martinson et al. 2012). The consumption of oxygen by bacteria in this region causes anoxic conditions. The bacteria also lower the pH and redox potential within the gut (Zheng et al. 2018). In contrast to the rest of the honey bee gut, the hindgut is lined with a stable cuticle. In the area connecting the midgut and hindgut, the pylorus, *Frischella perrara* is observed (Figure 1) (Engel et al. 2015). The ileum contains six longitudinal folds that are dominated by Gram-negative bacteria, specifically *Snodgrassella alvi*, a non-sugar fermenter (Figure 1). The centre of the lumen is dominated by *Gilliamella apicola*, a sugar fermenter (Figure 1) (Kwong and Moran 2013). Towards the end of the hindgut where faecal waste is stored, the rectum is dominated by Gram-positive *Lactobacillus* (Firm 4 and 5) and *Bifidobacterium asteroides* (Figure 1) (Olofsson and Vásquez 2008). The above-mentioned bacterial taxa are characteristically found in the gastrointestinal tract of honey bees across the world (Engel et al. 2012; Engel and Moran 2013; Kwong and Moran 2016), and gut microbes are most often passed from one individual to another through contact with adult bee faeces or the hive environment (Corby-Harris et al. 2014). Recent studies suggest the coexistence of these bacterial symbionts is largely possible due to resource partitioning, where competing taxa specialise on different nutrients (Brochet et al. 2021).

Bacteria are not the only microorganisms to be associated with honey bees and their gastrointestinal tract. A number of fungi could also contribute to the gut microbiota in significant ways. Most fungi associated with the honey bee gut are present in the hive environment (Nicolson et al. 2018; Yun et al. 2018). The most abundant of these, *Saccharomycetes*, are fermenters in bee bread (a mixture of pollen and nectar or honey) and the bee gut (Crotti et al. 2013). *Metschnikowia* are fermenters with slow growth, producing acid proteases. Dothideomycetes are often pathogenic, but their role in bee guts is not currently well known (Crotti et al. 2013). Less abundant fungi are also observed, such as Microbotryomycetes, Sordariomycetes, Tremellomycetes, and other rarer taxa (Crotti et al. 2013). In contrast to honey bee gut bacteria, the fungal diversity and abundance in bee guts are largely influenced by the environment, location, as well as food sources that bees are associated with (Crotti et al. 2013; Human et al. 2007). Unfortunately, the function and diversity of fungi associated with social bees is not well studied.

More recently, the presence of a virome within the guts of honey bees has been described (Bonilla-Rosso et al. 2020). The respective study identified 118 viral phage clusters (viruses that infect and replicate within bacterial and archaeal cells) that are, for the most part, genetically distinct from previously described phage genera. The fact that these phages were conserved across time and space, indicates that they are part of the core gut community of the honey bee. These phages could increase strain level variation within the honey bee gut microbiota when following the “kill the winner” hypothesis (Bonilla-Rosso et al. 2020; Cobián Güemes et al. 2016), thereby preventing one species from dominating the gut microbiome. The study was quite limited in its ability to detect viruses with single stranded

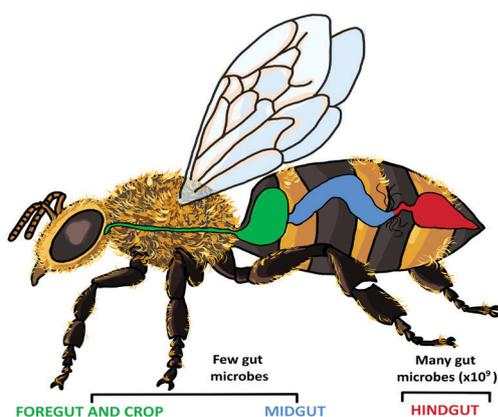


Figure 1: Main sections of the honey bee gut with the abundance of their respective microbes

RNA or DNA as well as small genomes, but still provides a starting point for future investigation. The true potential for the virome to harm or aid the insect host and gut community is still poorly understood. This work has opened new doors for studies on insect gut microbes, as viruses have historically been considered an infection that the host attempts to get rid of, or risk being compromised by a disease.

Most non-social insects have close associations with beneficial gut microbes. However, these individuals usually acquire microorganisms from the environment through horizontal transmission (Kikuchi et al. 2007). This has a decoupling effect on the coevolution of the host and symbiont, as the gut microbes need to retain larger genome sizes to survive outside of the host and invade host tissues at an appropriate time (Engel and Moran 2013). Some non-social insects facilitate vertical transmission of microbes by methods such as egg smearing, but this is not as common, as parent offspring contact is limited (Engel and Moran 2013). In contrast, vertical transmission of gut microbiota is a common characteristic among social insects (Engel and Moran 2013). This is due to the close association between individuals. For instance, honey bee workers typically stay within the hive environment tending to conspecifics for the first part of their lives. Social interactions have allowed social bees to evolve close associations with their characteristic gut microbial communities (these communities are consequently very conserved) (Figure 2) (Engel et al. 2012; Engel and Moran 2013). However, recent evidence suggests the hive environment (i.e., horizontal transmission) to be paramount in the acquisition of a healthy gut microbiome in honey bees (Anderson et al. 2023). These same bacterial taxa are consistently isolated in social bees across the world, however, they can be cultured *in vitro* (Zheng et al. 2018). Thus, this association is not so specific that the bacteria have lost functionality outside the host. Rather, the bacteria can establish and metabolise compounds outside of the bee gut (Engel and Moran 2013; Kwong et al. 2014).

Bumble bees (*Bombus* spp.) share similar gut symbionts commonly found in honey bees (*Apis* spp.) (Kwong et al. 2014; Martinson et al. 2011). More specifically, they share two core bacterial species, *Snodgrassella alvi* and *Gilliamella apicola* (Kwong et al. 2014). They also share a bacterial genus that is abundant in honey bees, *Lactobacillus* (Praet et al. 2015). However, the bacterial strains present in the honey bee gut cannot colonise the bumble bee gut and *vice versa* (Kwong et al. 2014; Martinson et al. 2011). When looking at all life stages and environments of the solitary European orchard bee (*Osmia cornuta*), Lozo and colleagues (2015) found their microbiome to be distinct from that of honey bees (Lozo et al. 2015). The authors

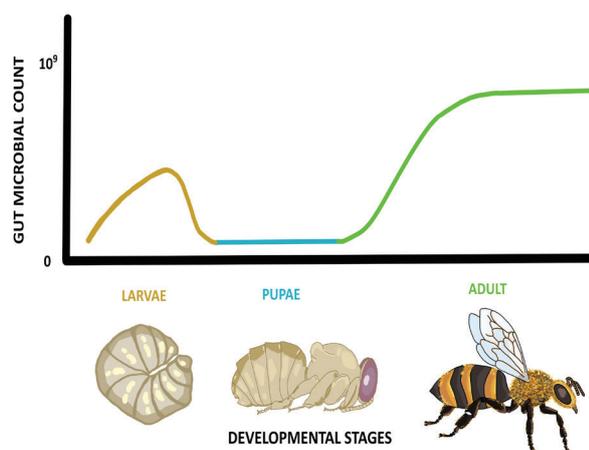


Figure 2: Change in the abundance of gut microbes throughout the development of the honey bee (adapted from (Kwong & Moran 2016)).

concluded that the solitary bee's bacterial diversity was derived from their environment (Lozo et al. 2015). This could be due to the lack of vertical transmission pathways in non-social species, where nests are isolated and interactions between conspecifics are limited (Kikuchi et al. 2007).

GUT MICROBIOME DIFFERENCES BETWEEN LIFE STAGES AND GROUPS OF HONEY BEES

The presence, structure and abundance of gut microbes within honey bees are not ubiquitous throughout their life cycle and differ between castes and sexes (Figure 2) (Kwong and Moran 2016; Martinson et al. 2012). As the larva emerges from the eggs their gastrointestinal tract is almost devoid of microorganisms (Martinson et al. 2012). The larvae are subsequently fed by nurse bees. This alloparental brood care provides larvae with microbes associated with nurse bees as well as the hive environment (such as their stored foods) (Martinson et al. 2012). During pupation, whilst the larva is transforming into an adult, the chitinous lining of the gut is shed and the pupal gut becomes sterilised. This process eradicates most microorganisms present in the honey bee gut (Martinson et al. 2012). Emerging adults will subsequently regain the full adult gut microbiota after four days, mainly through nest mates (faecal samples and trophallaxis) and hive materials (such as chewing the cell cap off as the adult bee emerges) (Anderson et al. 2023; Martinson et al. 2012). However, some studies suggest that the abundance and diversity of gut bacteria in workers decreases with age (Guo et al. 2015).

The gut microbial community of different worker bees (e.g., nursing and foraging bees) differs significantly (Yun et al. 2018), and the gut microbiota of drones and queens differ from that of workers (Figure 3) (Kapheim et al. 2015; Kwong and Moran 2016). Lastly, the gut microbial communities of honey bee larvae are more variable and decoupled from those of adults (Kowallik and Mikheyev 2021). *Lactobacillus* (Firm 4 and 5, Figure 3) are found more abundantly in the gut microbiota of the drones compared to that of workers. The reason for this is currently unknown. In contrast, the gut microbiota of the queen is dominated by *P. apium*, *Acetobacteraceae* and *Lactobacillus* (Firm 4 and 5). It is hypothesised that the discrepancy between the queen and worker gut microbiota might be due to a difference in diet (Kapheim et al. 2015). The increased occurrence of trophallaxis, grooming, and its effects on the queen gut microbiome also cannot be discounted.

EFFECTS OF MICROBES ON HONEY BEE NUTRITION

The most commonly studied aspect of the gut microbiota in all animals, is with regards to nutrition (Table 1) (Engel and Moran 2013). Without sufficient nutrient uptake by animals, they can no longer continue to survive and reproduce. Thus, nutrient uptake

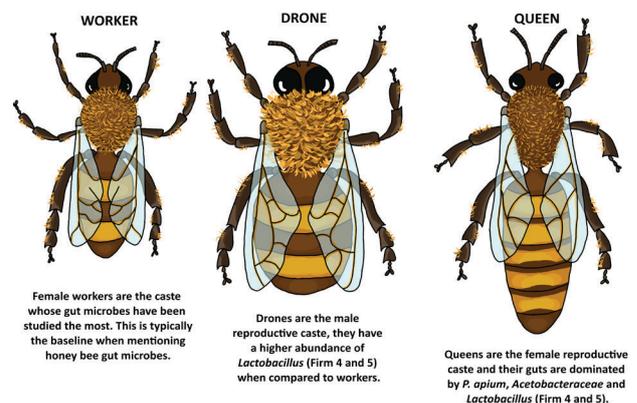


Figure 3: Differences in the gut microbes between castes and sexes (adapted from (Kwong & Moran 2016)).

is a strong driving force for evolution (Amato 2013). The most pronounced effects of the honey bee gut microbiome are studied by comparing gut-sterilised bees to those with gut symbionts (Zheng et al. 2017). These, typically general, methods have shown how important the gut microbiota are for successful functioning of an adult bee (Lee et al. 2015; Romero et al. 2019). The most striking results show that bees with a normal microbiome, gain weight 82% faster and attain a greater final body weight compared to individuals where the microbiota have been excluded (Zheng et al. 2017). Although survivorship was similar, adult bees deprived of gut microbes, had their weight gain halved (Zheng et al. 2017). This can have profound effects on the ability of an adult to conduct initial tasks as a nurse bee (Martinson

et al. 2012). Martinson et al. (2012) linked these observations to insulin signalling pathways. The authors determined that genes which produced certain peptides (*ilp1*, *ilp2*, *inR1* and *inR2*) associated with growth, reproduction, aging and nutrient homeostasis were upregulated (some, by a factor of 4.9–5.8). This is a result of amino acids produced by gut bacteria, increasing gene expression, and subsequently weight gain (Zheng et al. 2017). The absence of gut microbes is associated with a lower response to sucrose concentration, which ultimately affects bee satiation and weight gain (Bonilla-Rosso and Engel 2018). Gut microbes have also been shown to produce short chain fatty acids (SCFA), mainly acetate (Zheng et al. 2017). These SCFAs are also observed in humans and enhance the gut epithelial

Table 1: A summary of the recent literature pertaining to the honey bee gut microbiota, with particular focus on nutrition and immunity.

Category	Treatment/ method	Main effect	Proposed mechanism	Reference
Nutrition	Antibiotics and metabolomics	The symbiotic bacterium, <i>Bombella apis</i> , supplies larvae with essential amino acids such as lysine, which bolsters the larvae against weight reduction.	<i>B. apis</i> alters the amino acid content of honey bee larval diets.	Parish et al. 2022
	Antibiotic feeding trials.	Honey bees treated with antibiotics have impaired protein digestive efficiency.	Digestion of protein by the core gut microbiota of honey bees.	du Rand et al. 2020
	Plate assay with carbon substrates and Livak method.	Honey bee gut microbes express genes associated with utilisation of plant derived products and parts. They also produce a range of organic acids that the hosts may utilise.	Digestion of plant products by the gut bacteria that produce organic acids.	Lee et al. 2018
	Sterilised rearing of bees.	Honey bees with a typical gut microbiome experienced greater weight gain than bees with sterile guts.	Changes in the gut physicochemical conditions and microbial metabolites.	Zheng et al. 2017
	Plating pure cultures of <i>Gilliamella apicola</i> .	The authors isolated 42 strains of <i>G. apicola</i> from the honey bee gut and found that they could metabolise a wide range of toxic sugars produced by the breakdown of pollen.	<i>G. apicola</i> acquired both mannose PTS with a manO regulator through horizontal gene transfer.	Zheng et al. 2016
	Functional predictions through metatranscriptomics and metagenomics.	The three bacterial classes γ -Proteobacteria, Bacilli and Actinobacteria aid in carbohydrate digestion, digestion of fermentation products and the production of fermentation products. Ultimately, aiding in digestion of plant derived food.	Different metabolic processes conducted by the three main bacterial classes.	Lee et al. 2015
Immunity	Survival assay, antibacterial assay and transcriptomics.	Honey bees treated with antibiotics had a lower survivorship when infected with the pathogen <i>Hafnia alvei</i>	<i>Lactobacillus apis</i> was associated with the immune response by up regulating the production of AMPs.	Lang et al. 2022
	Sterilised rearing of bees and competition assays.	The pathogen <i>Serratia marcescens</i> persisted longer in honey bees with a disrupted microbiota, but was rapidly repelled in healthy bees.	Colonisation resistance through changes in the physicochemical properties of the gut.	Steele et al. 2021
	Comparison of infected and healthy individuals.	Honey bee larvae infected with <i>Paenibacillus larvae</i> (AFB) or <i>Ascosphaera apis</i> (Chalkbrood) had a disrupted gut microbiota. Specifically, they possessed fewer <i>Lactobacillus</i> and <i>Stenotrophomonas</i> bacteria, which may compromise the hosts immune response.	<i>P. larvae</i> produce secondary metabolites to kill or inhibit the growth of other bacteria.	Ye et al. 2021
	Antibiotics.	The exposure of antibiotics to honey bees increases antibiotic resistant genes (specifically tetB) and decreased their haemolymph antimicrobial abilities.	The reduction of <i>F. perrara</i> and <i>Lactobacillus</i> Firm-5 interfered with the immune response provided by the symbionts.	Daisley et al. 2020
	Sterilised rearing of bees.	Honey bees with disrupted gut microbes had a higher mortality rate and pesticide residue than those with healthy guts.	The expression of f P450 enzymes was reduced, compromising their detoxification pathways.	Wu et al. 2020
	Targeted proteomics with three treatment groups of bees.	Honey bees with a typical gut microbiota presented higher apidaecin concentrations and showed an improved survivorship when injected with <i>E. coli</i> .	The core gut symbionts produce an immune response, priming the immune system against pathogenic invaders.	Kwong et al. 2017
	Antibiotics.	Honey bees treated with antibiotics had a disrupted gut microbiota leading to a higher viral load of <i>Nosema ceranae</i> . Genes associated with producing AMP's (specifically abaecin, defensin1 and hymenoptaecin) were also down regulated.	Indirect host immune regulation through AMP's and direct antagonistic interactions between the gut symbionts and <i>Nosema ceranae</i> .	Li et al. 2017

barrier function. Those bees lacking microbes accumulated malate (a toxic sugar) and some important by-products were absent (Zheng et al. 2017). Gut microbes also aid in digestion of protein, as those bees treated with antibiotics had impaired protein digestive efficiency (du Rand 2020). It is important to note that antibiotics have been shown to affect host physiology as well, but the antibiotic used was specifically developed for honey bees and did not affect survival (du Rand 2020; Raymann et al. 2017). Taking this into account, it is clear that gut microbes significantly affect the physiology, metabolism, gene regulation and weight gain of adult honey bees (du Rand 2020; Zheng et al. 2017; Zhukova et al. 217).

While the benefits of a gut microbial community are clear, the role that each microbial taxon plays is less well understood (Zheng et al. 2018). Studying the specific function of each taxon is difficult as it is near impossible to exclude all except for one taxon from the bee gut, and the synergistic effects between microbes might go unnoticed (Zheng et al. 2018). However, metagenomics and the ability to culture these microbes makes the honey bee gut microbiome simpler to study. Metagenomic studies have investigated which bacterial taxa have genes associated with known functions (Engel et al. 2012). The approach used only provides an indication of the functions that might be present, by comparing gut microbial genes from the honey bee microbiome, against those gut microbes present in other species. *Gilliamella*, *Lactobacillus* and *Bifidobacterium* species possess genes associated with sugar transport functions (most abundantly of mannose) and are known to metabolise many different carbohydrates (Engel et al. 2012). Many sugars derived from pollen and nectar cannot be metabolised or are toxic (Zheng et al. 2016). Thus, the ability of gut bacteria such as *G. apicola* to digest toxic sugars like mannose, arabinose, xylose, or rhamnose is essential in the dietary health and tolerance of honey bees (Engel et al. 2012; Zheng et al. 2016). Subsequently, the above mentioned bacterial taxa aid in the breakdown of pectin, a major component of pollen cell walls (Engel et al. 2012). Pectin is toxic to honey bees, and the bacterial breakdown of this compound not only helps to release nutrients in pollen, but can also aid in avoidance of intoxication (Engel et al. 2012). Some bacterial taxa also have synergistic functions within the gut. For example, *Snodgrassella alvi* provides pyrimidines and amino acids to *G. apicola* (Kwong et al. 2014). The same study observed these taxa providing vitamins to one another as well.

By characterising the potential of a gut symbiont to fulfil a particular function through a number of genes, scientists are attempting to decipher the function of honey bee gut microbes. Unfortunately, there is still little known about the true function of the whole gut community in honey bees, thus, more research is needed. But it is clear that without this close association, honey bees would not have been as successful and numerous on most continents as they are today.

EFFECTS OF GUT MICROBES ON HONEY BEE IMMUNE FUNCTION

The direct effects that gut microbes have on host immune function is often overlooked due to difficulties with studying them (Emery et al. 2017; Kwong et al. 2017). However, exploring this topic is paramount for understanding honey bee immunity and how it relates to honey bee health (Table 1). A recent study looking at antimicrobial peptide (AMP) gene expression opened a new chapter on the effects gut symbionts have on gene expression and host immunity (Kwong et al. 2017). The genes they investigated commonly experience up-regulation in the honey bee haemolymph after bacterial infection (Aufauvre et al. 2014). The authors observed strong up-regulation of these genes associated with two AMPs, apidaecin and hymenoptaecin (Kwong et al. 2017). Bees with sterile guts exhibited lower

expression of AMPs and might be more susceptible to infection. The up-regulation of apidaecin and hymenoptaecin in the bee gut demonstrates the potential role of gut microbes in producing a systemic immune effect (Kwong et al. 2017). These AMPs could possibly play a part in the maintenance and homeostasis of the microbiome. They could also protect the host gut from invasion and colonisation by pathogenic microbes. Microbes associated with honey bees are tolerant to host AMPs and can subsequently persist in the host (Kwong et al. 2017). These results are critical in our understanding of honey bee immune function, but the experiments on AMPs are sensitive to growth condition. Subsequently, no significant difference in survival between bees with and without their gut microbiota were found when injected with *E. coli* (Kwong et al. 2017). Another study looked at antibiotic (tetracycline) treatment of adult worker bees and how this disruption increased their susceptibility to opportunistic pathogens. Honey bees with disrupted gut microbes were more susceptible and had higher levels of infection by *Serratia marcescens* (causing bee mortality) (Burritt et al. 2016; Raymann and Moran 2018).

American foulbrood (AFB) is a bacterial disease, caused by *Paenibacillus larvae*, that affects the honey bee brood (Forsgren et al. 2010). Many beekeepers struggle to control this disease with antibiotics leading to the disease causing significant economic damage (Raymann and Moran 2018). Recent outbreaks in South Africa have raised major concern with regard to the health of bee populations. However, novel studies have shown the key to fighting AFB might be found within the gut microbiome of honey bees (Forsgren et al. 2010). *Lactobacillus* and *Bifidobacterium* are identified, in tandem with the rest of the gut community, to inhibit the growth of *P. larvae* (*in vitro*) (Forsgren et al. 2010). The authors observed that the addition of the two symbionts significantly decreased the proportion of larvae infected with *P. larvae* that perished. This clearly shows that honey bees can resist AFB infections when gut health is optimal (Forsgren et al. 2010). Unfortunately, there are many factors that negatively influence the gut microbiota of honey bees (Forsgren et al. 2010). More recent studies have documented similar occurrences with other honey bee diseases. A study by Dosch and colleagues (2021) argued that the gut microbiota of honey bees increase their viral tolerance. In this study they exposed honey bees with sterile and healthy guts to the deformed wing virus (DWV) (Dosch et al. 2021). They concluded that the survival of honey bees with healthy guts was significantly higher than those without a healthy gut microbiome. The authors also highlight the fact that this significant effect was observed whilst both treatment groups possessed similar viral titres (i.e., the lowest concentration of a virus that still infects cells) (Dosch et al. 2021).

Although bumble bees (*B. terrestris*) do not possess the same gut microbiome as honey bees, they do share several species and are thus quite comparable (Koch and Schmid-Hempel 2011; Kwong et al. 2014). Recent studies on how gut microbes in bumble bees protect against an intestinal parasite, *Crithidia bombi*, further emphasise the role of gut microbes in host immunity (Koch and Schmid-Hempel 2011). Those bumble bees with native gut microbes had a parasite load one order of magnitude lower than those lacking gut symbionts. Thus, the native gut microbiota of bumble bees helps the host resist infection by parasites (Koch and Schmid-Hempel 2011). An investigation of microbial diversity in three bumble bee species infected and uninfected by *Crithidia*, showed that those infected had a lower abundance of *S. alvi* or *G. apicola* (Cariveau et al. 2014; Koch et al. 2012). Honey bees share a related parasite, *Crithidia mellificae*, and similar symbionts (Kwong et al. 2014; Langridge and McGhee 1967). Although the mechanism of this resistance is unclear, it may be that a similar trend will be observed in honey bees following research on this topic.

Some gut bacterial taxa do not always act symbiotically. *Frischella perrara* is known to cause scabs in the honey bee gut, and can act antagonistically (Engel et al. 2015). However, this bacterium can act symbiotically as it has also been linked to increased production of the AMP, apidaecin (Emery et al. 2017). Some rarer gut bacteria, such as *Serratia*, are linked with honey bee mortality and can be pathogenic (Raymann and Moran 2018).

FACTORS NEGATIVELY AFFECTING HONEY BEE GUT MICROBES AND POSSIBLE SOLUTIONS

Due to the recently recorded global decline of insect populations, more effort is being applied to conserve biodiversity (Halvorson et al. 2021; Sánchez-Bayo and Wyckhuys 2019). With regard to managed honey bees, recent evidence suggests that the risk of extinction is far less than wild colonies and other pollinators (Pirk et al. 2014; Requier et al. 2019). Managed western honey bees in particular comprise large populations globally due to their widespread use and breeding (Ellis 2012). However, surveys still suggest colony losses of more than 10% annually in 65 countries and 30% annually in 21 countries (Halvorson et al. 2021). This fluctuating population is problematic as honey bees are considered one of the most economically important insects worldwide, notably in the agriculture sector (Boncristiani et al. 2021; Gallai et al. 2009). They provide pollination services, thereby improving the yield, weight and genetic diversity of crops (Stein et al. 2017). Honey bees are also managed for their honey production, and subsequently produce precious agricultural products worth \$150 million annually in the US alone (National Agricultural Statistics Service). Unfortunately, disease, poor nutrition, pesticides and pollution are emerging prominent predicaments, causing colony losses of up to 65% in some countries (Genersch 2010). This has sparked bee keepers and farmers to adopt desperate strategies in order to control honey bee diseases and pests.

For susceptible honey bee populations in-hive pesticides containing chlorothalonil, coumaphos (CheckMite+) and fluvalinate (Apistan) are often used to control *Varroa* mites and other honey bee parasites (Kakumanu et al. 2016). These chemicals are used in close contact with honey bees and are sold as being safe for bees. However, recent studies have documented that all of the tested chemicals, most notably chlorothalonil, significantly affected the gut bacterial community structure of honey bees (Kakumanu et al. 2016). Specifically, chlorothalonil significantly lowered the abundance of *Lactobacillus* and *Bifidobacterium*, with other gut bacteria taking their place and the specific functioning of the gut bacteria is altered, decreasing sugar metabolism and protease activity (Kakumanu et al. 2016). Ultimately, this decreases the nutrients gained through the mutualistic relationship between honey bees and their gut bacteria. In addition, the immune function of bees decreases with disruptions in their typical gut bacterial community (Kwong et al. 2017). Earlier, we specifically discussed how associations with *Lactobacillus* and *Bifidobacterium* protect honey bees from pathogens and parasites. A decrease in the abundance of these two important gut bacterial genera could be devastating to honey bee health, physiology and nutrition. Effects of in-hive insecticides on gut fungi are not clear, as the environment where the hives were placed played a larger role in fungal diversity (Kakumanu et al. 2016). With the physical ramification of in-hive pesticides on honey bee mortality as a whole, it is clear that these chemicals can do more harm than good (Kakumanu et al. 2016; Traynor et al. 2016).

Recent evidence suggests that honey bees contaminated with typical agricultural pesticides are also at risk (Motta et al. 2020; Steinigeweg et al. 2023; Traynor et al. 2016). A study investigating pesticide residues on migrated honey bee hives found 171 different

pesticide residues present in the wax, bee bread and adult workers (Traynor et al. 2016). The authors found that colonies exposed to more pesticide residues experienced much higher mortality rates, especially when pollinating agricultural crops, as most pesticide exposure is experienced near farms. Interestingly, the authors concluded that the presence of particular fungicides in the hive wax is correlated with colony mortality. Chemicals from insecticides that are deemed “bee safe” have also been isolated and correlate with increased colony mortality (Traynor et al. 2016). A recent study by Almasri et al. (2022) reported that exposure of honey bees to environmentally realistic concentrations of herbicides, insecticides and fungicides caused disruption to their metabolism and detoxification systems. The authors found that the gut microbiome of the exposed honey bees remained largely intact. However, individuals with disrupted gut microbial communities suffered larger negative effects compared to healthy honey bees. Indicating that their core gut microbiota aid in the physiological resilience of honey bees against pesticide exposure (Almasri et al. 2022). Other studies have documented how moderate to high doses of pesticides disrupt the gut microbiome of honey bees (Al Naggar et al. 2022; Motta et al. 2018; Motta et al. 2020; Zhu et al. 2020). Therefore, one moderate pesticide exposure event near honey bees could potentially hinder their ability to resist future chronic exposure to pesticides, even at lower concentrations. Recent microbial pest control products, which aim to reduce non-target pesticide toxicity, have also fallen short as an alternative (Steinigeweg et al. 2023). Steinigeweg et al. (2023) showed that the microbial pest control product, *Bacillus thuringiensis*, increased honey bee brood termination rate and caused a significantly lower abundance of core gut bacterial species.

Although the risks associated with pesticides on non-target insects are well studied, less attention has been given to the secondary effects this can have on the gut microbiome (Goulson et al. 2015; Kakumanu et al. 2016). Some pathogens, such as AFB, cause extensive economic damage to managed honey bee hives. This calls for the use of antibiotics to reduce the extensive economic damage associated with these pathogens (du Rand 2020; Human et al. 2011). However, these compounds (like in-hive pesticides) were developed and put into use before the essential functions performed by gut microbes were known. Although antibiotics affect the gut microbial composition, these seemingly insignificant changes can have negative consequences for honey bees (du Rand 2020; Raymann et al. 2017). Antibiotics not only affect protein digestion, but increase honey bee mortality and susceptibility to diseases and opportunistic microbes (du Rand 2020; Raymann et al. 2017). Antibiotic resistance in symbiotic gut microbes can lead to horizontal gene transfer between symbiotic and antagonistic species (du Rand 2020; Raymann et al. 2017), making these diseases even more difficult to control.

South Africa has legislation in place to minimise honey bee mortality due to pesticides. Agricultural remedies used in South Africa have to adhere to strict regulations under the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act 36 of 1947. However, honey bee mortality due to pesticides is still commonly observed around the country (Pirk et al. 2014). The main culprit is the unlawful use of remedies, as well as the sub-lethal effects these chemicals can have. This includes using chemicals that have not been approved or thoroughly tested by the registrar and/or not following product label instructions.

Furthermore, many beekeepers adhere to guidelines outlined in other countries where AFB is controlled with antibiotics (Human et al. 2011). This is unsustainable, as resistant strains of AFB have been observed in the United States, United Kingdom and South America, to name a few (Cunningham et al. 2022; Evans 2003). Most countries, South Africa included, have

no registered antibiotics to use against AFB. However, trace amounts of antibiotics were present in honey from treated hives in countries where antibiotics were banned (Ghose and Hawkins 2004; Human et al. 2011). Some beekeepers in South Africa have resorted to extreme measures such as using antibiotics associated with chickens to treat AFB (Ezette du Rand, personal communication). This is not limited to AFB, as concoctions of undisclosed chemicals have been used to treat numerous infected honey bee colonies (Abdullahi Ahmed Yusuf and Ezette du Rand, personal communication).

Thankfully, the above-mentioned practices are limited to a small group of mostly commercial beekeepers in South Africa (Ezette du Rand, personal communication). African honey bees are considered very resilient against pathogens and parasites (Dietemann et al. 2009; Pirk et al. 2016). Thus, correct hive management, prevention and control practices are recommended to treat diseases affecting African honey bees (Dietemann et al. 2009; Human et al. 2011; Pirk et al. 2016). When we look at AFB specifically, infected colonies should be destroyed. However, for most honey bee diseases, beekeeping equipment and hives should be sterilised, hives should not be situated close to each other and contact with other apiaries should be avoided (Human et al. 2011).

Honey bees and their respective gut microbes could also experience pressures on a global scale (Harvey et al. 2023). Climate change has already affected many species and will affect even more in future (Karl and Trenberth 2003). The interactions between environment, hosts, symbionts and pathogens are still largely ambiguous within honey bees. It is likely that a rise in global temperatures could pose a serious threat to honey bees and their gut microbes (Cunningham et al. 2022; Harvey et al. 2023; Kikuchi et al. 2016). A recent study has shown how the gut microbiome of non-social insects died out when temperatures were raised by 5 °C above normal (Prado et al. 2010). Kikuchi and colleagues (2016) concluded that the gut symbiotic association between insect and bacteria collapse during simulated climate change temperatures 2.5 °C higher than ambient. Although these studies are alarming, honey bees can regulate their hive temperature and might be more resistant to such effects (Cunningham et al. 2022; Fahrenholz et al. 1989). However, no study has attempted to shed light on the effect that rising global temperatures has on the gut microbiome of social bees.

AREAS OF FUTURE RESEARCH and CONCLUSION

The effects of gut microbes on animals have only recently gained attention (Amato 2013; Kuziel and Rakoff-Nahoum 2022). The field is new and expanding, and there are still many unanswered questions. Most studies have focused on bacteria, as these associations are more stable and structured compared to other microorganisms (Kwong and Moran 2016; Zheng et al. 2017). Although, gut bacteria are the main focus of most studies, the full range of species and strains present in gut communities is still unknown, as are the functions these microbes perform. In the honey bee gut, which is considered simple, we do not know all the effects that microbes have on individual behaviour, physiology, nutrition and immune function. There has been a wide range of methods used to study the bee gut microbiome, and these methods help us understand different parts of the same system. Unfortunately, not enough work has been done to elucidate all the functions gut microbes accomplish. Thus, there is a need to continue investigating the effects that gut microbes have on honey bee gene expression and metabolism. Advancements in genetics, metabolomics (the study of all metabolites and chemical processes relating to metabolites within an organism) and metatranscriptomics (the study of gene expression within microbial communities) have permitted the study of gut microbes with greater power, but new and improved

methods are required to understand the full extent of how gut microbes influence the host (Kwong and Moran 2016). Most studies are laboratory based. Although field studies are more difficult to control, they are recommended, as this will provide real-world knowledge of how microbial interactions shape honey bee fitness.

The effects of gut fungi and protozoa on honey bees have not yet been well documented. These associations are not as fixed and depend greatly on the environment. However, recent studies have shown greater colony mortality when fungicide residuals were present in bee hives (Traynor et al. 2016). Therefore, overlooked associations between the importance of gut fungi in honey bees need to be evaluated. The significant effect of fungicides on colony losses could be a result of hive-associated fungi being affected. Nevertheless, it is important to investigate all possible negative associations. Antagonistic protozoa and viruses, such as nosema, have been identified in honey bees (Genersch 2010). Yet some insects, for example termites, have symbiotic associations with protozoans (Lee et al. 1987). Similarly, the gut virome, which increases gut microbial diversity within the honey bee, has been identified. Thus, it could be fruitful to identify protozoan or virus species associated with the honey bee gut and identify their functions.

Lastly, the effects of pollutants (such as heavy metals, carbon dioxide, nitrogen dioxide, pesticides, etc.) on the gut microbial structure of honey bees should be further investigated (Figure 4) (Goulson et al. 2015; Leita et al. 1996; Lusebrink et al. 2015). Studies have observed how “bee safe” pesticides can negatively affect honey bees (Traynor et al. 2016). However, recent evidence indicate a healthy gut microbiome could be key to pesticide resistance (Wu et al. 2020). Subsequently, with more knowledge of what affects honey bee microbes, strategies can be implemented to limit honey bee exposure to these chemicals.

More research into probiotics are needed, as previous studies have shown the importance of these gut microbes on the successful functioning of honey bees (Engel et al. 2012; Kwong and Moran 2016). Current commercially available probiotics seem to be ineffective at best, and antagonistic at worst (Ptaszyńska et al. 2016). Fascinatingly, honey bee propolis has shown some evidence that it might bolster the core gut microbiome of honey bees (Saelao et al. 2020). However, a plethora of new research indicates the potential of probiotics to bolster the immunity of honey bees (Elzeini et al. 2021; Maruščáková et al. 2020; Motta et al. 2022; Powell et al. 2021).

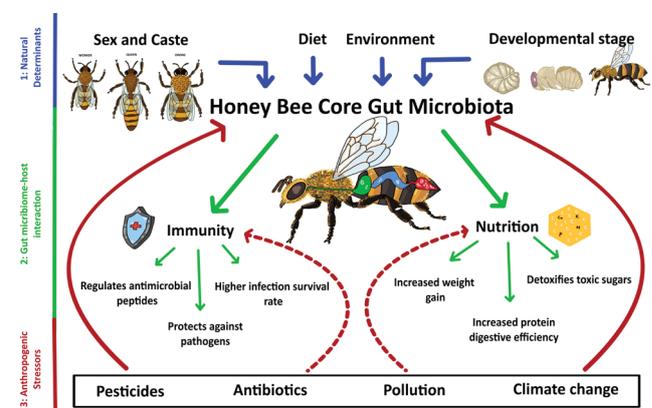


Figure 4: Flow diagram of the interactions between the honey bee gut microbiome (2), natural determinants (1) and anthropogenic stressors (3). The blue arrows represent natural determinants that influence the gut microbiome (1), the green arrows are positive functions provided to the host (2). The solid red arrows represent direct negative impacts on the gut microbiome and the dotted red arrows represent indirect negative effects that impact the host due to the disruption of the gut microbiome (3)

By improving our current understanding of the bee microbiota, effective probiotics are likely to be developed to further reduce honey bee losses, especially due to phenomena affecting the honey bee gut microbiome (Elzeini et al. 2021; Maruščáková et al. 2020; Motta et al. 2022; Powell et al. 2021).

It is clear that the gut microbiota of honey bees is essential for their continued functioning and are in part responsible for their widespread success (Figure 4). Four bacterial taxa, *Lactobacillus*, *Bifidobacterium*, *S. alvi* and *G. apicola* are the best studied and research suggests they have a disproportionate effect on nutrition and immune function in honey bees. Disruption of this core microbiome can lead to disastrous effects. There is, undoubtedly, much we still do not fully grasp regarding the honey bee gut microbiota. However, with new methods, uncovering the functions of these microbes is more feasible. With this additional knowledge, flaws are revealed in the ways we protect our honey bees. Antibiotics and in-hive pesticides that are used to benefit bees have been shown to cause greater harm than thought (Figure 4). Thus, a more sustainable approach, including through the development and use of probiotics, is needed to achieve better honey bee health.

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